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# PCB-Induced Inhibition of the Vesicular Monoamine Transporter Predicts Reductions in Synaptosomal Dopamine Content

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**Both Aroclor mixtures and individual non-coplanar polychlorinated biphenyl (PCB) congeners reduce dopamine (DA) concentrations in cells in culture and in the brains of developing and adult laboratory animals. These reductions may involve inhibition of the dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT) responsible, respectively, for the uptake of extracellular DA and the packaging of nerve terminal cytosolic DA into synaptic vesicles. However, the relative contribution of each monoamine transporter to the PCB-induced reductions in tissue DA has not been determined. Accordingly, we exposed striatal synaptosomes from adult rats to individual PCB congeners, a commercial mixture of PCBs or known monoamine transporter inhibitors; measured synaptosomal DA; and related these changes to media DA and concentrations of 3,4-dihydroxyphenylacetic (DOPAC). PCB-induced elevations in media DA concentrations are not sufficient to explain the reductions in tissue DA because known DAT inhibitors elevate media DA to a much greater extent than PCBs and yet induce similar decreases in tissue DA concentrations. On the other hand, PCB-induced elevations in DOPAC, reflective of increases in nerve terminal cytosolic DA, are sufficient to explain the reductions in tissue DA, because a known VMAT inhibitor elevates DOPAC and reduces tissue DA to an extent similar to that seen with PCBs. Taken together, these results suggest that elevations in DOPAC, reflective of increases in nerve terminal cytosolic DA due to VMAT inhibition, rather than elevations in media DA due to DAT inhibition, are largely responsible for the observed decreases in tissue DA content.**

*Key Words:* **polychlorinated biphenyls (PCBs); dopamine; synaptosomes; dopamine transporter (DAT); vesicular monoamine transporter (VMAT).**

Using both *in vivo* and *in vitro* techniques, Seegal and colleagues have demonstrated that polychlorinated biphenyls (PCBs) alter dopamine (DA) function, including reductions in tissue DA content and elevations in extracellular DA concentrations in rat brain (Seegal *et al*., 1991, 1994, 2002) and organotypic cultures of striatal tissue (Bemis and Seegal, 1999; Chishti *et al*., 1996).

One mechanism by which PCBs may mediate these changes is via inhibition of the plasma membrane DA transporter (DAT) (Mariussen *et al*., 2001a,b; Rosin and Martin, 1981), which is responsible for the re-uptake of extracellular DA. Interestingly, these, as well as other *in vitro* effects, including activation of protein kinases (Kodavanti *et al*., 1993) and alteration in intracellular calcium (Wong and Pessah, 1996), are only seen following exposure to non-coplanar PCB congeners. Thus, PCB-induced DAT inhibition, and the consequent elevations in extracellular DA, may activate presynaptic autoreceptors that, in turn, influence both the synthesis and further release of DA into either media or the extraneuronal space (Wolf and Roth, 1990).

PCBs, including both individual congeners and commercial mixtures of congeners, also inhibit the vesicular monoamine transporter (VMAT) (Mariussen *et al*., 2001a), which packages nerve terminal cytosolic DA into synaptic vesicles (Nirenberg *et al*., 1996). Inhibition of VMAT function increases 'free' nerve terminal cytosolic DA (Teng *et al*., 1997; Zetterstrom *et al*., 1988). In turn, elevations in nerve terminal cytosolic DA may lead to end-product inhibition of tyrosine hydroxylase (TH) (Cerrito and Raiteri, 1980; Kumer and Vrana, 1996) and formation of reactive oxygen species (ROS) (Miller *et al*., 1996) shown to reduce DA synthesis and content (Cooper *et al*., 1996; Minami *et al*., 1992).

Thus, there is considerable evidence that both non-coplanar PCB congeners and commercial mixtures of PCB congeners inhibit monoamine transporters which, in turn, play an important role in regulating DA function. Indeed, Gainetdinov and Caron (2003) demonstrated profound changes in DA function in DAT knockout mice. However, the relative contributions of PCB-induced inhibition of DAT and VMAT function in influencing tissue DA content have not been determined. In the experiments described below, we determined changes in media DA and DOPAC, recognized functional surrogates, respectively, for DAT and VMAT inhibition (Garris *et al*., 2003; Lee *et al*., 2001; Mazei *et al*., 2002), in order to determine the consequences of PCB-induced inhibition of these transporters on synaptosomal DA content and compared these changes with those induced by known monoamine transporter inhibitors.

## MATERIALS AND METHODS

***Selection of PCB congeners.*** PCBs were selected for study based on their ability to differentially alter DAT and VMAT function. These values,

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**TABLE 1**
**PCBs Differ in Their Ability to Inhibit Monoamine Transporter Function**

| PCB | Structure | DAT $IC_{50}$ (μM) | VMAT $IC_{50}$ (μM) |
|---|---|---|---|
| C95 | 2,3,6,2′,5′ | **1.2**[a] | nd |
| C91 | 2,3,6,2′,4′ | nd | 5 ± 0.3[b] |
| Aroclor 1254 | | 3.7 ± 0.2[c] | nd |
| C153 | 2,4,5,2′,4′,5′ | >50[c] | 14 ± 3.3[b] |
| C103 | 2,4,6,2′,5′ | 14.7 ± 0.2[c] | **7 ± 1.0**[b] |

[a]Seegal Laboratory.

[b]Mean ± standard deviation (Mariussen *et al*., *Toxicol. Appl. Pharmacol.* **175**, 176–183, 2001.

[c]Mean ± standard error of the mean (Mariussen and Fonnum, *Toxicology* **159**, 11–21, 2001.

[c]Bold=IC50 values fail to predict the reductions in synaptosomal DA observed.

summarized in Table 1, are derived from Mariussen *et al*. (2001a,b) with the exception of the 2,3,6,2′,5′-pentachlorobiphenyl (C95) DAT values that were determined in our laboratory. We also examined the coplanar congeners, 3,4,3′,4′-tetrachlorobiphenyl (C77) and 3,4,5,3′,4′-pentachlorobiphenyl (C126). These latter congeners were selected as negative controls since these are typically inactive in altering DA function (Mariussen *et al*., 2001a,b; Shain *et al*., 1991) and regulating intracellular calcium (Kodavanti *et al*., 1993; Wong and Pessah, 1996) in *in vitro* systems.

***Preparation of PCB solutions for exposure.*** PCB congeners (3,4,3′,4′-tetrachlorobiphenyl (C77); 2,3,6,2′,4′-pentachlorobiphenyl (C91); 2,3,6,2′,5′-pentachlorobiphenyl (C95); 2,4,6,2′,5′-pentachlorobiphenyl (C103); 3,4,5,3′,4′-pentachlorobiphenyl (C126); and 2,4,5,2′,4′,5′-hexachlorobiphenyl (C153)) were purchased from Accustandard (New Haven, CT; >99% purity); Aroclor 1254 (A1254) was obtained from the Wadsworth Center, New York State Department of Health, and was analyzed by high-resolution mass spectrometry (O'Keefe *et al*., 1985) for polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs). Total PCDFs were 22.4 ppm and no PCDDs were found at the detection limit of 0.3 ppb.

All PCBs were dissolved in dimethylformamide (DMF) to create 1000X concentrated stock solutions that were then used to prepare media containing PCBs at the concentrations used in this study (0–40 μM). An approximate molecular weight of 326.4 g/mol (Erickson, 1997) was used to calculate the estimated molarity of the A1254 solution.

***Preparation of synaptosomes.*** The isolation of purified striatal synaptosomes is based on a modification of procedures described by Löscher and coworkers (1985). Briefly, 10–12 week-old male Long-Evans rats (Taconic Farms, Germantown, NY), were stunned and decapitated and their brains were rapidly removed. The following procedures were performed at 4°C unless otherwise noted. A forebrain block, made by a coronal cut anterior to the optic chiasm, was isolated and the striata dissected free-hand. Striata from 6–8 rats were pooled in ice-cold 0.32 M sucrose prior to homogenization with a Potter-Elvehjem glass-Teflon tissue grinder (0.15–0.23 mm clearance). The resulting homogenate was centrifuged at 1000 × *g* for 10 min, and the supernatant collected and layered onto 1.2 M sucrose. Centrifugation at 50,000 × *g*, with an $\omega^2 t$ setting of $1.6 \times 10^{10}$ yielded an interface layer, which was collected, diluted, layered onto 0.8 M sucrose and centrifuged for a second time, using the same conditions as described above to yield the purified synaptosomal pellet. Synaptosomal pellets were resuspended in a volume of oxygenated HEPES-buffered Hank's saline (HBHS) equivalent to the starting wet weight of the striatal tissue and kept on ice until use. All experiments were performed with the approval of the Wadsworth Center Institutional Animal Care and Use Committee.

***Exposure of synaptosomes and preparation for neurochemical analysis.*** Thirty μl aliquots of synaptosomes were then suspended in 750 μl of HBHS containing 1% horse serum and either 0.1% DMF or 0.1% DMF + PCBs (2.5, 5, 10, 20, or 40 μM), distributed into 96-well plates (130 μl/well) and incubated for 30 min in a humidified shaking water bath under an atmosphere of 95% $O_2$ /5% $CO_2$ at 37°C. After exposure, the samples were transferred to microcentrifuge tubes and centrifuged for 5 min at 12,000 rpm to separate the synaptosomes from the medium. 100 μl of the resulting supernatant was removed and acidified by the addition of a volume of 0.4 N $HClO_4$ equal to the volume of the supernatant, while the synaptosomal pellet was homogenized in 100 μl of 0.2 N $HClO_4$. All samples were frozen at −80°C until analysis was performed, generally within 1–3 days.

Experiments were also performed to determine the effects of two DAT inhibitors, nomifensine (NOM) and GBR 12935 (GBR) (Garris *et al*., 2003; Lee *et al*., 2001; Mazei *et al*., 2002), both from Sigma (St. Louis, MO), and a VMAT inhibitor RO 4–1284 (Jones *et al*., 1998) (a gift from Hoffman-La Roche, Nutley, NJ) on synaptosomal tissue and media DA and DOPAC concentrations. Aqueous stocks of these agents were serially diluted in media to the appropriate test concentrations and DMF was added to each solution to result in a final DMF concentration of 0.1%.

***High performance liquid chromatographic analysis of DA and DOPAC.*** Quantification of synaptosomal and media DA and DOPAC concentrations was performed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) as described previously (Bemis and Seegal, 1999; Chishti *et al*., 1996). Neurotransmitter concentrations were corrected for synaptosomal protein content determined by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL). The protein concentration in each well was approximately 200 ng/ml. Total DOPAC concentrations (media + tissue) were reported since DOPAC is actively transported across the plasma membrane (Lamensdorf *et al*., 2000) and summation of the changes in both compartments provides the best estimate of the ability of PCBs or RO 4–1284 to inhibit VMAT-mediated uptake of nerve terminal cytosolic DA.

***Measurement of synaptosomal integrity.*** Synaptosomal plasma membrane integrity was estimated by measuring media lactate dehydrogenase (LDH) activity, since LDH release is a recognized measure of disruption of synaptosomal plasma membrane integrity (Berman *et al*., 1997). Briefly, following exposure of synaptosomes to PCBs, the medium was collected and the synaptosomes were disrupted ultrasonically to liberate intra-synaptosomal LDH. LDH release into media was expressed as a percentage of total LDH (media + intra-synaptosomal) to correct for the total number of synaptosomes. This method was performed with a modified Sigma Diagnostics LDH Assay® based on procedures described by Amador and coworkers (1963).

***Statistical analyses.*** Neurochemical data (expressed as percent of DMF control) examining the effect of treatment of synaptosomes with increasing doses of PCB and media LDH activity (expressed as percent of total LDH activity) were analyzed using 1-way analysis of variance (ANOVA). Bonferroni-corrected *post hoc t*-tests allowed comparison of differences between treatment groups (mean ± SEM). Correlations between alterations in media DA or total DOPAC concentrations and synaptosomal DA concentrations were examined using linear regression analyses. Results are based on 2–3 replicate experiments with 5–6 wells per experiment.

## RESULTS

### *PCBs Differ in Their Abilities to Reduce Synaptosomal DA Concentrations*

We statistically compared the abilities of PCBs to alter synaptosomal DA concentrations by determining the concentration of the congener or mixture of PCBs (expressed on a micromolar basis) that would result in a 25% reduction in synaptosomal DA. We chose this metric, determined from the complete dose-response curve for each congener or mixture at 30-min exposure, because many of the PCBs, even at the highest concentration,

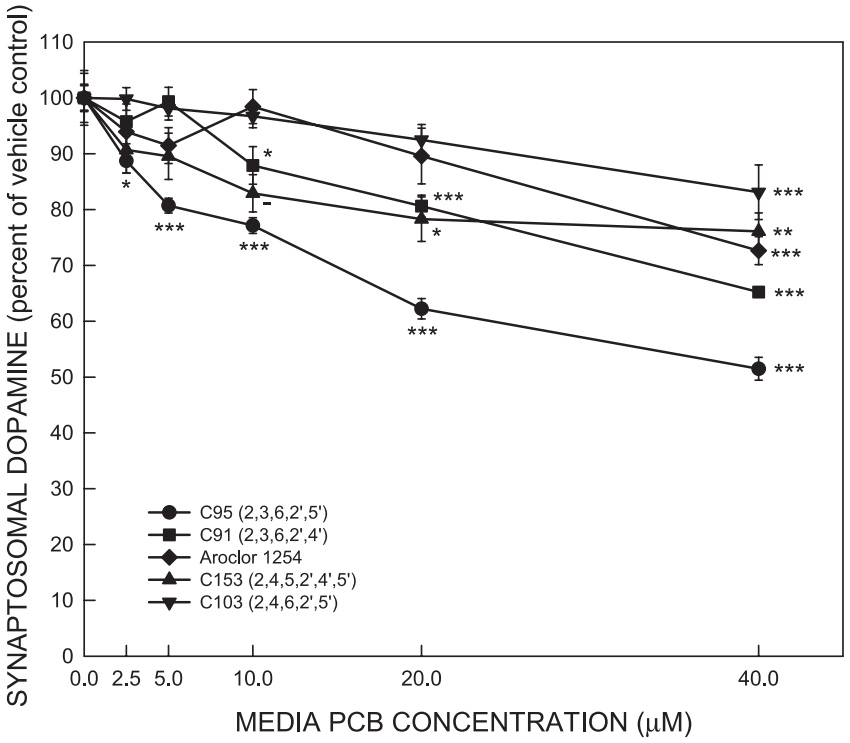


**FIG. 1.** Effects of 30-min exposure of striatal synaptosomes to media containing vehicle (0.1% dimethylformamide [DMF]) or 2.5, 5, 10, 20, or 40 μM concentrations of PCBs on synaptosomal dopamine concentrations. Overall significance of dose response of effects of individual PCBs: C95 (F = 88.14; *df* = 5,54; $p \leq 0.001$); C91(F = 25.11; *df* = 5,51; $p \leq 0.001$); Aroclor 1254 (F = 6.14; *df* = 5,54; $p \leq 0.001$); C153 (F = 4.76; *df* = 5,52; $p \leq 0.01$); C103 (F = 8.90; *df* = 5,100; $p \leq 0.001$); $n$ = 9–18 observations per treatment condition (mean ± SEM). ($-p \leq 0.1$, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$; significance of *post-hoc* *t*-tests comparing individual concentrations against the vehicle control.)

tested (40 μM), did not reduce DA concentrations by 50%, thereby obviating the more classical $IC_{50}$ measurement. Additionally, we chose not to use PCBs at concentrations greater than 40 μM, because preliminary data (not shown) demonstrated, for many of the congeners, loss of synaptosomal membrane integrity as measured by large increases in LDH release.

PCB congeners differed in their abilities to reduce synaptosomal DA concentrations (Fig. 1, Table 2). C95 was the most active congener ($EC_{25}$ = 11 μM), followed by C91 ($EC_{25}$ = 27 μM). The $EC_{25}$ values of A1254 and C153 were similar to one another (37 and 40 μM, respectively). C103 was the least active of the non-coplanar PCBs tested with an $EC_{25}$ significantly greater than 40 μM. Neither coplanar PCB congener altered tissue DA concentrations (data not shown).

**TABLE 2**
**Effects of PCBs and Transporter Inhibitors on Neurochemical End Points at the $EC_{25}$ for Reductions in Synaptosomal Dopamine**

| | Synaptosomal dopamine $EC_{25}$ (μM) | Media dopamine (% Control) | Total DOPAC (% Control) |
|---|---|---|---|
| C95 | 11 | 123 | 127 |
| C91 | 27 | 123 | 117 |
| A1254 | 37 | 354 | 105 |
| C153 | 40 | 139[a] | 108[a] |
| C103 | ≫40 | 179[a] | 105[a] |
| GBR 12935 | 127 (nM) | 625 | 90 |
| Nomifensine | 7 (μM) | 694 | 88 |
| RO 4–1284 | 7 (nM) | 74 | 114 |

[a] Determined at 40 μM concentration.

### *PCBs Differ in Their Abilities to Elevate Media DA Concentrations*

All non-coplanar PCBs elevated media DA concentration, albeit with differing potencies (Fig. 2, Table 2). A1254, at 40 μM, induced the largest increase in media DA concentrations (to approximately 350% of the control value), followed by C95, which increased media DA to approximately 200% of the control value. C103 increased media DA concentrations to approximately 150% of the level seen in controls, while the remaining congeners increased media DA concentrations by less than 25%. The co-planar congeners C126 and C77 did not alter media DA (data not shown).

### *PCB-Induced Elevations in Media DA Do Not Correlate with Reductions in Synaptosomal DA*

Despite the fact that all PCBs increased media DA concentrations, the correlations between PCB-induced elevations in media DA and reductions in synaptosomal DA concentrations

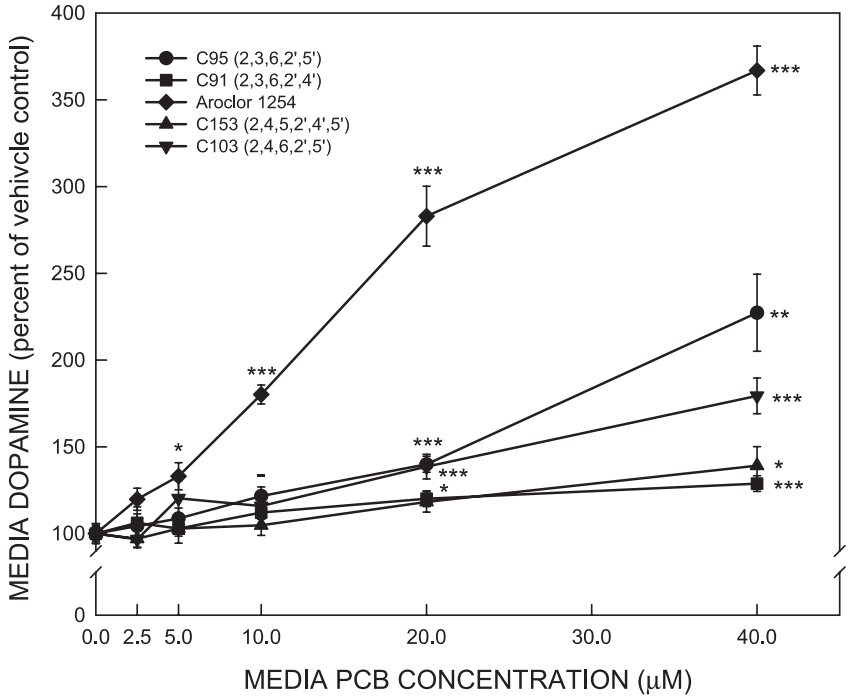


**FIG. 2.** Effects of 30-min exposure of striatal synaptosomes to media containing vehicle (0.1% DMF) or 2.5, 5, 10, 20, or 40 μM concentrations of PCBs on media dopamine concentrations. Overall significance of dose response of effects of individual PCBs: C95 ($F = 22.49$; $df = 5,53$; $p \leq 0.001$); C91($F = 4.06$; $df = 5,52$; $p \leq 0.01$); Aroclor 1254 ($F = 101.46$; $df = 5,54$; $p \leq 0.001$); C153 ($F = 5.02$; $df = 5,53$; $p \leq 0.001$); C103 ($F = 16.41$; $df = 5,99$; $p \leq 0.001$); $n = 9$–18 observations per treatment condition (mean ± SEM). ($-p \leq 0.1$, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$; significance of *post-hoc t*-tests comparing individual concentrations against the vehicle control.)

(all at 40 μM) were not statistically significant. Either using all of the data, or excluding the A1254 data because of the large increases in media DA concentrations induced by that PCB mixture, we determined the $r^2$ to be 0.0057 or 0.26, respectively, indicating a poor relationship between these dependent variables (data not shown).

### *PCBs Differ in Their Abilities to Elevate Total DOPAC Concentrations*

Statistically significant elevations in total DOPAC were observed following exposure to C91 and C95: 40 μM concentrations of these congeners resulted in maximal elevations of DOPAC to 118 and 128% of control, respectively. C103, C153, A1254, and the coplanar congeners C77 and C126 did not significantly alter DOPAC concentrations (Fig. 3; Table 2).

### *Elevations in Total DOPAC Concentrations Correlate with Reductions in Synaptosomal DA*

PCB-induced reductions in synaptosomal DA concentrations and PCB-induced elevations in total DOPAC concentrations were highly correlated ($r^2 = 0.89$; Fig. 4). These results suggest that elevated nerve terminal cytosolic DA (estimated as total DOPAC) may significantly inhibit DA synthesis and/or lead to enhanced metabolism of DA.

### *Effects of PCBs on LDH Release*

Exposure of synaptosomes to PCBs, under conditions identical to those used for the collection of the neurochemical data, revealed no significant elevations in media LDH activity for any of the PCBs examined (data not shown).

## DISCUSSION

The reductions in synaptosomal DA following exposure to PCBs, as well as the relationship between the congener structure and the ability to reduce DA concentrations, are similar to those findings reported for striatal slices exposed *ex vivo* to PCBs (Bemis and Seegal, 1999; Chishti *et al*., 1996); for pheochromocytoma (PC12) cells (Shain *et al*., 1991) and for bovine adrenal chromaffin cells (Messeri *et al*., 1997). Most importantly, reductions in synaptosomal DA content and elevations in media DA and total DOPAC were seen following exposure to PCBs at concentrations similar to those detected in the brains of postnatal-day-21 rats exposed during gestation and lactation to Aroclor 1254 (Crofton *et al*., 2000).

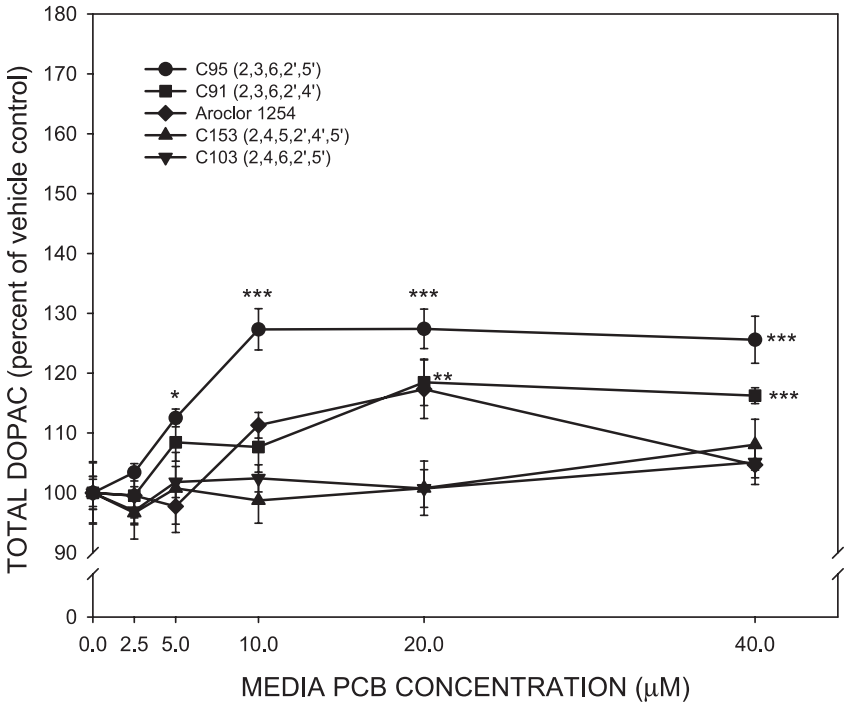


**FIG. 3.** Effects of 30-min exposure of striatal synaptosomes to media containing vehicle (0.1% DMF) or 2.5, 5, 10, 20, or 40 μM concentrations of PCBs on total DOPAC (synaptosomal + media) concentrations. Overall significance of dose response of effects of individual PCBs: C95 ($F = 18.48$; $df = 5,54$; $p \leq 0.001$); C91($F = 5.45$; $df = 5,50$; $p \leq 0.001$); Aroclor 1254 ($F = 3.61$; $df = 5,54$; $p \leq 0.01$); C153 ($F = 0.65$; $df = 5,51$; $p = 0.66$); C103 ($F = 1.10$; $df = 5,98$; $p = 0.36$); $n = 8$–18 observations per treatment condition (mean ± SEM). (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; significance of *post-hoc t*-tests comparing individual concentrations against the vehicle control.)

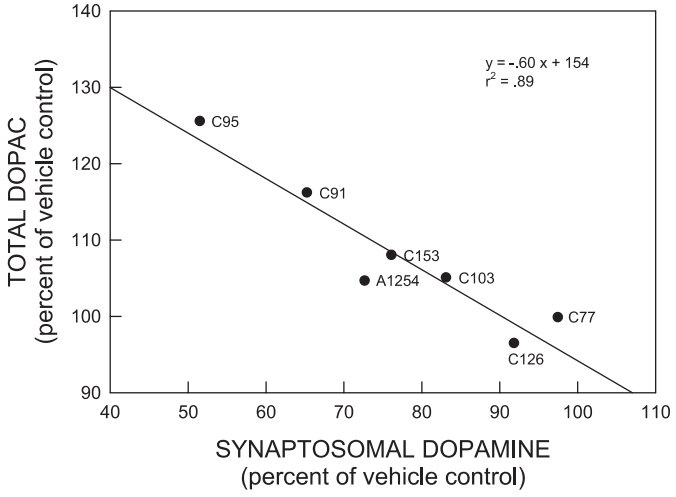


**FIG. 4.** Statistical relationship between means for total DOPAC and synaptosomal dopamine concentrations in striatal synaptosomes following 30-min exposure to media containing PCBs at 40 μM concentrations, determined using linear regression analysis ($F = 39.622$; $df = 1,5$; $p \leq 0.001$); $n = 9$–18 observations per PCB congener.

The mechanisms responsible for the decreases in tissue DA, however, are not fully understood. In a previous study, where we observed significant reductions in tissue DA concentrations in striatal slices exposed *ex vivo* to PCBs (Bemis and Seegal, 1999), we suggested that elevations in media DA concentrations, due to inhibition of DAT function (Mazei *et al*., 2002), are responsible for the reductions in striatal DA content. This hypothesis, that PCBs influence tissue DA concentrations by inhibiting DAT function, was supported not only by findings of Rosin and Martin (1981) but also by recent findings from Mariussen and Fonnum (2001b). Both groups demonstrated that either Aroclor mixtures or non-coplanar PCB congeners significantly and competitively inhibited uptake of labeled DA into whole brain synaptosomes. The data presented here, however, suggest that, at least for those congeners which result in the greatest reductions in tissue DA content, elevations in media DA play a less important role in reducing synaptosomal DA concentrations than do elevations in nerve terminal cytosolic DA due to PCB-mediated inhibition of the VMAT. This hypothesis is based on three findings.

First, there is only a weak statistical relationship between PCB-induced elevations in media DA and reductions in synaptosomal DA concentrations. This lack of a statistically significant relationship suggests that PCB-induced inhibition of DA uptake and the subsequent reductions in the amount of DA available for re-packaging into storage vesicles has only a minor effect on synaptosomal DA content.

Second, the elevations in media DA concentrations following exposure to NOM and GBR, associated with a 25% reduction in synaptosomal DA, are much greater than the elevations in media DA following exposure to PCBs (Table 2). These discrepancies in the levels of media DA associated with similar reductions in

synaptosomal DA suggest that, except perhaps for A1254, PCBs do not elevate media DA to levels sufficient to significantly influence synaptosomal DA content and that other mechanisms must be evoked to explain the reductions in synaptosomal DA.

Finally, we have previously demonstrated that A1254 significantly elevated extra-neuronal DA (indicative of PCB-induced DAT inhibition) without significantly altering rat striatal DA concentrations (Seegal *et al*., 2002). These latter results, obtained using *in vivo* microdialysis, support our contention that DAT inhibition, resulting in elevations in extra-neuronal DA concentrations, at least in the short term, plays at best only a minor role in influencing striatal DA levels *in vivo*.

The data for PCB-induced VMAT inhibition (and subsequent elevations in nerve terminal cytosolic DA) in reducing synaptosomal DA concentrations are considerably stronger. The greatest reductions in DA were observed following exposure to either C95 or C91, both of which significantly elevated total DOPAC concentrations (Fig. 4), indicative of an elevation in free nerve terminal cytosolic DA (Teng *et al*., 1997). In turn, elevations in nerve terminal cytosolic DA may ultimately influence synaptosomal DA by a number of mechanisms, including reductions in *de novo* DA synthesis by end-product inhibition or activation of presynaptic receptors, and enhanced metabolism of nerve terminal cytosolic DA to quinones and/or sulfated metabolites (Kumer and Vrana, 1996).

This hypothesis, that PCBs reduce synaptosomal DA concentrations by elevating nerve terminal cytosolic DA, is supported by two additional pieces of evidence. First, the concentration of RO 4–1284 associated with a 25% reduction in synaptosomal DA concentration, elevated total DOPAC to a level nearly identical to that seen following exposure to either C91 or C95, the two congeners that reduced synaptosomal DA to the greatest extent (Table 2). Second, the correlation between PCB-induced elevations in total DOPAC and reductions in synaptosomal DA is statistically highly significant, suggesting that PCB-induced elevations in free nerve terminal cytosolic DA are responsible for the decreases in synaptosomal DA concentrations.

The PCB congeners examined here, and the majority of those reported by Fonnum and coworkers, may inhibit DA uptake at both monoamine transporter sites. However, we suggest that VMAT inhibition (and the resulting elevation in nerve terminal cytosolic DA, seen here as elevations in total DOPAC) is most likely responsible for the reductions in synaptosomal DA following exposure to C95 and C91. Elevations in nerve terminal cytosolic DA are less likely to be responsible for the reductions in DA following exposure to A1254 where inhibition of DAT function appears to be the primary mechanism responsible for the relatively modest decreases in synaptosomal DA. However, the remaining PCB congeners, which were unremarkable in their ability to alter either DAT or VMAT function, may involve low-level inhibition of both transporters.

Additional mechanisms may also contribute to the observed reductions in synaptosomal DA including ROS formation, due to metabolism of increased nerve terminal cytosolic DA concentrations, alterations in intrasynaptosomal calcium concentrations (Wong et al., 1997) as well as nonspecific PCB-induced structural modifications of cytoplasmic membranes. Indeed, Kim *et al*. (2001) demonstrated structural changes, including increased membrane fluidity, in *Ralstonia eutropha* H850 membranes following long term exposure to the non-coplanar congener 2,5,2′,5′-tetrachlorobiphenyl. It is unlikely, however, that PCB-induced changes in transcription contribute to the reductions in synaptosomal DA because of the rapidity with which changes in synaptosomal DA occur.

Finally, an examination of the data in Table 1 provides a cautionary note concerning the relationship between monoamine transporter inhibition and alterations in synaptosomal DA concentrations. These data, particularly for the C95 and C103 values that are shaded, demonstrate that measurement of PCB-induced inhibition of uptake of labeled DA may fail to predict the reductions in synaptosomal DA induced by some PCBs. For example, although A1254 and C95 have similar DAT $IC_{50}$ values, they differ significantly in their ability to elevate media DA concentrations. On the other hand, the $IC_{50}$ for C103 VMAT inhibition is similar to that for C91, despite the relatively small increase in total DOPAC seen with C103. These discrepancies may reflect the fact that PCBs, unlike highly specific pharmacological transporter inhibitors, may influence both transporters.

In addition to reducing synaptosomal DA concentrations, PCB-induced inhibition of VMAT, resulting in elevated levels of 'free' nerve terminal cytosolic DA, may also reduce neuronal viability. In this study, however, we found no evidence of loss of membrane integrity. Nevertheless, elevated concentrations of free nerve terminal cytosolic DA (shown here as an increase in total DOPAC concentrations) have been shown to act as an endogenous neurotoxin (Berman and Hastings, 1997; Montine *et al*., 2000) that induces oxidative stress, impairs protein and membrane functions, and modifies DNA/RNA (Berman and Hastings, 1999; LaVoie and Hastings, 1999). Thus, PCB-mediated alterations in intracellular DA storage may exacerbate the neurodegeneration occurring in normal conditions (e.g., aging), as well as interacting with other environmental risk factors (e.g., rotenone) and genetic predispositions associated with certain disease states, e.g., Parkinson's disease (De Iuliis *et al*., 2002).

In summary, the evidence presented here strongly suggests that PCB induced elevations in free nerve terminal cytosolic DA, rather than increases in either media or extra-neuronal DA, play the greatest role in reducing tissue DA concentrations and perhaps, in the long term, leading to changes in behavior and/or initiating or enhancing neurodegenerative processes associated with Parkinsonism.

## ACKNOWLEDGMENTS

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